CENTER FOR INDOOR AIR RESEARCH

1099 WINTERSON ROAD
SUITE 280
LINTHICUM, MD 21090
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APPLICATION FOR RESEARCH CONTRACT

1. PRINCIPAL INVESTIGATOR. Name, title, telephone # and mailing address.

(a) Hanspeter R. Witschi (Name) (b) Professor (Title) (c) 916-752-0915 (Telephone number)

(d) VM:Pharmacology/Toxicology (Department) (e) University of California, Davis (Institution)

(f) Toxic Substances Program, University of California (Mailing Address) (g) CA 95616 (State/Zip)

2. PROJECT TITLE. (Do not exceed 75 typewriter spaces inclusive of spaces between words and punctuation.)

Neuroendocrine Lung Cancer: Mechanistic Studies

3. KEY WORDS. Please provide three (3) key words which will be used as reference headings.

Cancer (lung); neuroendocrine cells; tumor promotion

4. INSTITUTION. Name and address of institution responsible and accountable for disposition of funds awarded on the basis of this application.

(a) Regents of the University of California (Institution) (b) 275 Mrak Hall (Street Address)

(c) Davis (City) (d) CA 95616 (State/Zip)

5. LOCATION. List location where research will be conducted *if other than institution identified in #4 above.*

(a) N/A

(b)

6. INCLUSIVE DATES and TOTAL COSTS of this specific project related to each 12 month period if more than one yea is required to complete project. Summarize from budget page, item 13(j). It must be understood that awards for 2n and 3rd periods are dependent on Science Advisory Board review and Center approval of continuation applicatior

	Inclusive Date			Total Cost
(a) 1st 12 month period if required	9/1/90	thru	8/31/91	$ 166,099
(b) 2nd 12 month period	9/1/91	thru	8/31/92	$ 169,159
(c) 3rd 12 month period	9/1/92	thru	8/31/93	$ 179,200

7. INSTITUTIONAL OFFICER. Name, title and telephone # of individual authorized to sign for the institution identifie in #4 above. It is understood that the officer, in applying for a contract, has read and found acceptable the Center Management of Research Contracts and Contract Administration Policy.

(a) Name (b) Title

(c) Telephone (d) Signature of institutional officer (e) Date

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13. BUDGET. Detail specific needs for first 12-month period. Estimate category sub-totals for 2nd and 3rd periods, if required. Append justifications.

(a) Salaries. List personnel by name and title. Indicate individuals % time to be spent on this project.	$ 1st period	$ 2nd period	$ 3rd period
% Professional:			
30 Hanspeter R. Witschi, Professor	REDACTED		
* Jerold A. Last, Professor (no salary requested)			
100 Victor Oreffo, Research Associate			
Technical:			
50 Laboratory Technician			
100 Histology Technician, SRAI			
Other:			
30 Clerical Support (purchasing/secretarial)			
Fringe benefits payable at institution's rate of 33** %			
Category (a) Sub-Total	$ REDACTED		
(b) Consultants (per diem, travel & expenses):			
Charles Plopper, Professor (no salary requested)			
Fred Meyers, Professor (no salary requested)			
Category (b) Sub-Total	$ 0	$ 0	$ 0
(c) Supplies & Expense:			
Consumables (by category)			
Biochemical reagents and histology supplies, including reagents for immunochemistry	17,500		
Animals and related costs			
200 Syrian Golden Hamsters	1,100		
Animal maintenance	13,912		
Other expenses (itemize)			
IEHR use fee (1% TDC)	1,298		
Radioactive & hazardous waste disposal	1,200		
Service contracts on equipment	2,000		
Publication	500		
Telephone, xerox, office supplies	1,000		
Category (c) Sub-Total	$ 38,510	$ 40,821	$ 43,270
(d) Travel & Expenses:	1,500	1,500	1,500
Category (d) Sub-Total	$ 1,500	$ 1,500	$ 1,500
(e) Alterations and Renovations			
Category (e) Sub-Total	$ 0	$ 0	$ 0
(f) Sub-contracts			
Category (f) Sub-Total	$ 0	$ 0	$ 0

* as required
** based on average of actual benefit rates

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Category (g) Sub-Total	$ 8,300	$ 2,000	$ 2,120
(g) Equipment			
Polymerase Chain Reaction Machine, Cetus	8,300		
Miscellaneous small equipment (repair & replace)		2,000	2,120
(h) TOTAL DIRECT COSTS	$139,362	$140,836	$149,196
(i) Indirect costs not to exceed 25% of the sum of (a) thru (f):	$ 26,737	$ 28,323	$ 30,004
(j) TOTAL PROJECT COSTS	$166,099	$169,159	$179,200

14. BIOGRAPHICAL SKETCH of all professional personnel listed in 13(a). Append. Please include the following: Name title, education, scientific field, major research interest, research and/or professional experience and publications (Limit list of publications to the 20 most important and/or relevant.)

See attached

15. a) Are HUMAN SUBJECTS to be used in this research? _____ Yes ___X___ No

If yes, attach Institutional Review Board approval for procedures involving human subjects.

b) Are LABORATORY ANIMALS to be used in this research? ___X___ Yes _____ No

If yes, attach Institutional Animal Care and Use Committee approval for procedures involving animals.

See attached

16. SIGNATURE OF PRINCIPAL INVESTIGATOR. It is understood that the applicant in applying for a Contract ha read and found acceptable the Center's "Statement of Policy and Terms Under Which Project Contracts Are Made.

_____ Date_____

Signature of Principal Investigator

rev. 2/89

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Title of project: Neuroendocrine Lung Cancer: Mechanistic studies

Investigators: H.P. Witschi

Institution: University of California, Davis

Abstract

Lung cancer is the most frequent cause of death from cancer in man. There are two major forms of human lung cancer: small cell and non-small cell cancer. The successful prevention and treatment of lung cancer will eventually be based on a knowledge of the molecular and cellular events underlying the pathogenesis of this disease. Understanding of oncogene expression is a crucial step in such a development. We propose to analyze oncogene expression in non-small cell lung cancers induced in hamsters by a lung-specific nitrosamine and in small cell lung cancers produced by concomitant exposure of hamsters to a nitrosamine and hyperoxia; treatment regimens will be used that induce tumors within a comparatively short time period (3 to 4 months) and with high incidence (70% to 80%). Hamster-specific probes will de developed and oncogene expression will be evaluated during tumor development and in the tumors that eventually form. We hypothesize that oncogene expression (ras, myc) and/or tumor suppressor inactivation (Rb, p53) is an obligatory early event in development of lung tumors. Thus, we predict that there will be characteristic patterns of oncogene expression for both tumor types to be studied herein. These early patterns of gene expression will allow for accurate prediction of ultimate tumor type.Analysis of neuroendocrine cell proliferation in lung tissue of hamsters exposed to a carcinogenic regimen will serve as an additional quantitative and qualitative indicator of lung tumor development.

Signature, Principal Investigator

5/29/1990
Date

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Research Plan

8. Specific aims

Lung cancer is the largest single cause of death among all cancer deaths (Doll and Peto, 1981; Bunn, 1988). In man, lung cancer can be divided into two major forms: small cell lung cancer and non-small cell lung cancer; the differentiation is important with regard both to prognosis and therapeutic approaches. It is now possible to produce both forms of lung cancer with high incidence and within a short induction time in experimental animals. Modern techniques have revealed multiple and potentially important differences in the molecular biology of the two major lung cancer forms in man. However, the significance of these findings with regard to biological behavior of the tumors such as slow or rapid progression, metastatic potential or amenability to chemotherapy remains unclear. Some of these questions regarding the correlation between molecular biology and clinical behavior of lung tumors now need to be addressed experimentally in studying animal models of lung cancer. We have the animal models available and propose to pursue the following specific aims:

1. To correlate the expression of specific oncogenes with subsequent development of small cell and non-small cell lung cancers in hamsters. Specifically we want to address the question of whether oncogene expression could be used as an early indicator of the nature of tumors that will develop. Comparative studies will be done with dominant-acting proto-oncogenes (ras and myc) and repressor proto-oncogenes (retinoblastoma, rb and p53).

2. To examine oncogene expression in the resultant lung tumors in order to see whether maintenance of the tumor state requires continuous expression of oncogenes.

3. To test the hypothesis that, as a consequence of altered oncogene expression, we can use quantitative and qualitative differences in pulmonary neuroendocrine cell hyperplasia as a further predictor of tumor development.

We hypothesize that oncogene expression (ras, myc) and/or tumor suppressor inactivation (Rb, p53) is an obligatory early event in development of lung tumors. Thus, we predict that there will be characteristic patterns of oncogene expression for both tumor types to be studied herein. These early patterns of gene expression will allow for accurate prediction of ultimate tumor type.

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9. Significance of proposed work

a) Background

Lung cancer in man

Human lung cancers can be subdivided into two major forms: Non-small cell cancers and small cell cancers. Adenocarcinomas represent the major leading type of non-small lung cancer today whilst the incidence of squamous cell carcinoma has decreased (Wynder et al., 1985). Nitrosamines have been used to induce adenomas and adenocarcinomas in the lungs of laboratory animals (Reznik-Schuller, 1983; Hillfrich et al., 1977). Schuller (1989) has suggested that the recent rise in pulmonary adenocarcinoma in man may be simply attributable to the predominance of nitrosamines as carcinogenic stimuli in cigarettes. Of this group of compounds, NNK, formed from the nitrosation of nicotine, has been found to be one the most potent lung carcinogens known in laboratory animals(Hecht and Hoffmann, 1988).

Small cell lung cancer originates from pulmonary neuroendocrine cells. The different forms of neuroendocrine lung cancer range from the relatively benign carcinoids to the most malignant form, small cell lung cancer. The cell of origin is the Kultchitsky (APUD cell of the epithelium lining the airways. Small cell cancer of the lung, the most malignant variety, accounts for approximately 30% of all pulmonary cancers (Iannuzzi and Scoggins, 1986).

Molecular biology of lung cancer

The prevention and treatment of any lung cancer will eventually be based on a knowledge of the molecular and cellular events underlying the pathogenesis of this disease. Understanding of oncogene expression is a crucial step in such a development. In normal healthy cells, oncogenes exist as so-called proto-oncogenes and are involved in self regulating systems of cell growth and cell differentiation (Bishop, 1983). The current thinking on the molecular origins of cancer is that normal proto-oncogenes, present in all cells, can be converted into cancer causing oncogenes by carcinogens. Thus, activated oncogenes may arise as a result of point mutations, chromosomal translocations, deletions or gene amplification. Genetic lesions can involve the activation of dominant acting cellular proto-oncogenes (Bishop, 1987) and the inactivation of recessive "tumor suppressor" genes (Klein, 1987). These lesions may act together to bring about the transformation of lung epithelial cells (Minna et al., 1986). The dominant acting oncogenes that appear to have a role in human lung cancer belong to two families of closely related genes, ras and myc.

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Activated ras oncogenes have mainly been encountered in lung cell lines of non-small-cell origin ((Wong et al., 1986). The genes code for a family of 21 kilodalton (Kd) guanosine triphosphate-binding proteins (p21), that are related to G proteins and are thought to play a part in signal transduction. It has been suggested that ras proteins are involved in growth-stimulatory, signal-transduction pathways from the outer cell membrane to the interior of the cell (Heldin and Westermark, 1984). The ras oncogenes acquire the ability to transform cells from the normal to tumor phenotype by a point mutation within the protein (Tabin et al., 1982; Reddy et al., 1982). These activating mutations result in substitutions of different amino acids in the 12th, 13th, or 61st codons of p21. There is evidence that known carcinogens can cause point mutations in ras genes of animal tumors (Barbacid, 1987). Evidence exists that there is a correlation between expression of oncogenes with exposure of patients exposed to carcinogens such as tobacco smoke (Rodenhuis et al., 1987, 1988). The group of tumors studied included among others adenocarcinoma, squamous or epidermoid cell carcinoma, and large cell carcinoma (collectively known as non-small cell lung cancer). Mutations were found only in adenocarcinomas. The incidence was about 30% and nearly all mutations were found in K-ras codon 12. In these results, a positive relation between the smoking history of patients and the presence of a mutated ras gene was found, suggesting that a mutagenic component in tobacco smoke may cause the ras mutation.

Myc genes code for nuclear proteins involved in growth regulation (Eisenman et al., 1985). The myc oncogene, in contrast to the ras, is useful as a prognostic marker in examining neoplasms for gene amplification. Myc family members (C-myc, N-myc, and L-myc) have been reported to be expressed in both small cell and non-small cell lung cancer. C-myc amplification has been explored in patients with lung cancer (Johnson et al., 1987). It was found that patients bearing c-myc amplified tumors have a poor prognosis compared with other extensive SCLC. Studies which address myc oncogene expression would be useful in terms of grading tissue transformation. A number of studies have shown that complete transformation of a normal cell into a malignant cell involves the activation of more than one oncogene (Minna, 1984). It has been shown that an activated ras gene together with an activated myc gene can cause malignant transformation. It is likely that several mutation events need to occur in the same lung cell before a tumor can develop and metastasize (Land et al., 1983).

The retinoblastoma (Rb) gene belongs to a class of genes known as "tumor suppressor", "recessive oncogenes", or "anti-oncogenes". Tumor formation or transformation occurs when such genes are inactivated. This suggests that their normal function is to limit cellular proliferation. The main difference between a dominant oncogene (e.g. ras, myc) and a recessive oncogene is that for the former, it is the presence of the product, whereas for the latter,

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it is the absence of the product that leads to transformation. Tumor suppressor genes have been linked to lung cancer; for example loss of DNA in chromosome 13 has been localized to the Rb locus (Harbour et al., 1988). It has been shown that a large number of SCLC in humans have absent or reduced expression of the Rb gene product (Friend et al., 1986). A recently discovered anti-oncogene is the cellular protein p53. Evidence that p53 is an anti-oncogene came from the finding of p53 deletions in several mouse erythroleukemic cell lines (Mowat et al., 1985). The p53 transforming potential is apparently due to inactivation of a normal wild type p53 activity. p53 mutants may function in transformation by acting as dominant inhibitory proteins that block the activity of wild type endogenous p53. The p53 gene is frequently mutated or inactivated in all types of human lung cancer, and its disruption appears to play an important role in the pathogenesis of human lung cancer (Takahashi et al., 1989).

Tumorigenesis can occur as a result of overexpression of a cellular proto-oncogene, or its altered expression in a given cell may lead to transformation. Alternatively, the lack of expression of a specific cellular gene(s) or inactivation of their product(s) may also play a vital role in cancer development. Ultimately, it appears to be the combination of all these events that is required in tumorigenesis.

Lung cancer in animals

Animal models are indispensable if we wish to correlate molecular biology with in-vivo tumor development, progression and response to therapy. For the study of non-small cell lung cancer, several animal models are available. Adenocarcinomas are easily induced in hamster lungs by treatment with several nitrosamines (Reznik-Schuller, 1983). Squamous cell carcinomas can be produced with polycyclic aromatic hydrocarbons (Saffiotti et al., 1968). As far as small cell lung cancer is concerned, it only recently became possible to produce the equivalent of this specific lung cancer in experimental animals. Three years ago the first animal model of neuroendocrine lung cancer was described (Schuller et al., 1988); the model is representative of human small cell lung cancer. In a more recent study, Schuller et al. (1990) have shown that the nitrosamine NNK can be used to produce both SCLC and non-SCLC in hamsters. Induction time for both types of tumors is comparatively short (3 to 6 months) and over-all tumor incidence is high (>70%). We thus have the possibility to study the two major tumor types in the same animal species.

Because of being a recent development, induction of neuroendocrine lung tumors in hamsters needs to be described briefly. In Syrian Golden Hamsters exposed to an atmosphere of 70% oxygen for 8 weeks (a treatment the animals tolerate well), a multiple hyperplasia of pulmonary neuroendocrine cells developed

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in all animals. Multiple neuroendocrine cell hyperplasia was also found in hamsters kept in room air and treated, for 8 weeks, twice a week with subcutaneous injections of diethylnitrosamine (DEN). In neither of the two groups did we find tumors after 8 weeks. However, in hamsters kept in 70% oxygen and simultaneously given DEN injections (twice a week for 8 weeks) a different picture evolved. While in the oxygen atmosphere, the animals began to lose weight (a phenomenon not seen in hamsters kept in oxygen alone or given DEN treatment in air only) and some animals, showing extreme cachexia had to be killed before 8 weeks. At autopsy we found multiple tumors in the lungs of all animals (i.e. tumor incidence was 100%).

Histopathological analysis, electron microscopy and specific immune stains showed unequivocally that the tumors were of neuroendocrine cell origin: they contained the typical electron-dense granules and stained positive for bombesin, calcitonin and neuron-specific enolase. A combined exposure to hyperoxia and DEN thus produced tumors of neuroendocrine cell origin within a very short time period and with a very high incidence. Although the tumors did not resemble in all features typical human SCLC, it nevertheless could be concluded that we actually had developed the first animal model of one of the most important forms of human lung cancer.

As do neuroendocrine lung tumors in hamsters, human SCLC also appear to arise from the neuroendocrine cells (Kultschitzky cells, APUD cells) found in the epithelium of the airways (Gould et al., 1983). Neuroendocrine cells are rarely found in the lungs of adult animals, but occur in abundance in the lungs of fetal and newborn animals. The cells are believed to play a role in helping the lung to adjust to changes in the oxygen content of the inspired air. Histochemical evidence shows that the cells contain vasoactive peptides such as bombesin, a growth factor for neuroendocrine cells, as well as serotonin and calcitonin which act as broncho- or vaso-dilators or -constrictors. Neuroendocrine cells act as chemoreceptors; they respond to hyperoxia and hypoxia in the lung (Schuller, 1988). In patients suffering from chronic obstructive lung disease, their number can be increased as it is in people living at high altitudes (Becker, 1984). Acute or chronic hypoxia will dramatically increase the number of neuroendocrine cells in the airways of rats and rabbits (Pack et al., 1986; Taylor, 1977; Keith and Will, 1981; Moosavi et al., 1973). More recently it was found that daily exposure to smoke produced from a single standard research cigarette (7 minutes of exposure) for 90 days produced a measurable increase in the number of pulmonary neuroendocrine cells (Tabassian et al., 1989).

A possible direct link between neuroendocrine cell proliferation and tumor development was documented in our previous studies (Schuller et al., 1988, 1990). In a serial sacrifice study we found that after only a few weeks in hyperoxia, cells reacting

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positive for mammalian bombesin appear in the alveolar parenchyma. This indicates that some alveolar type II cells (which begin to proliferate in hyperoxia as witnessed by an increased incorporation of radiolabelled thymidine into DNA) may also transdifferentiate into neuroendocrine cells. The lung now contains an increased, actively proliferating and expanding cell population of neuroendocrine cells. The cells are capable of taking up and metabolizing nitrosamines. They also may secrete increased amounts of the peptide bombesin, a growth factor for neuroendocrine cells. It is our current working hypothesis that such a combination of events then leads to rapid tumor development (Schuller, 1988). It follows that increased proliferation of pulmonary neuroendocrine cells might serve as a marker for eventual tumor development.

Gaps in Research Area

We propose to analyze the molecular biology of non-small cell and of small cell lung cancer in a hamster model. The major gap in the research are is the absence, to date, of any published data on oncogene expression in hamster lung. The second major gap is the absence of any numerical data on neuroendocrine cell proliferation during pathogenesis of neuroendocrine lung cancer. For example, it is not known at the time being whether the observed apparent increase in neuroendocrine cells is caused by division of neuroendocrine cells or by transdifferentiation of other cell types in the lung to neuroendocrine cells (Schuller et al., 1990). Also it is not known why neuroendocrine cell proliferation caused by nitrosamines alone (Reznik-Schuller, 1976) does not lead to the development of neuroendocrine tumors.

Significance of research

Lung cancer is the leading cause of death from cancer. It will only be through an eventual understanding of the molecular and cellular events of the disease that we will be in a position to effectively prevent or to cure it. In addition, by being in a position where we both know and can quantitate mechanisms, we will be capable eventually of predicting the risk for lung cancer from exposure to polluted atmospheres, be they indoor or outdoor air.

We have also performed a series of preliminary experiments examining agents other than DEN and 70% oxygen for their ability to produce neuroendocrine lung tumors in experimental animals under controlled experimental conditions. As discussed under preliminary studies, Polonium-210 can be substituted for DEN or 0.8 ppm of ozone for 70% of oxygen in the hamster model and neuroendocrine lung tumors are still elicited within a few months at a high level of incidence. In additional work not discussed in this proposal we have also examined three other agents capable of causing

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neuroendocrine cell proliferation for their ability to replace hyperoxia in our tumor model: hypoxia, nitrogen dioxide and carbon monoxide. Based upon preliminary findings (which do not yet include rigorous histopathologic identification of cells with specific immunestains) all of these later compounds have the potential to produce neuroendocrine cell hyperplasia and, eventually, lung tumors of neuroendocrine cell origin in the lungs of hamsters treated with DEN or NNK. Thus, several indoor air pollutants have the potential (at very high concentrations as compared to those actually encountered in indoor air) to influence lung tumorigenesis in hamsters: nitrogen dioxide, carbon monoxide, ozone and Polonium-210 (a radon decay product). It is beyond the scope of this proposal to further explore these findings in vivo, but they speak to the potential usefulness and relevance of our model to concerns about indoor air pollution.

10. PRELIMINARY STUDIES

Feasibility of proposed research

Our initial studies on the induction of pulmonary neuroendocrine lung tumors in hamsters have been published (Schuller et al., 1988, 1990). In work done at UC Davis during the last year (1989) we have confirmed the original observations. In addition, we have gathered preliminary data which further strengthen our hypothesis that neuroendocrine cell proliferation in the respiratory tract of hamsters is an obligatory event leading to neuroendocrine lung tumor development. Our observations may be summarized as follows.

Multiple tumors of neuroendocrine cell origin could be produced in hamsters exposed to hyperoxia while being given, once a week, an intratracheal instillation of the alpha-emitting radioisotope Polonium-210 (0.2 uCi per instillation, a total of 8 instillations). Polonium-210 is well known for its capability to induce adenocarcinomas and squamous cell carcinomas in hamster lungs (Little and O'Toole, 1974; Little and Kennedy, 1979). We now have shown that hyperoxia-induced neuroendocrine cell proliferation favors the development of radiation-induced neuroendocrine lung tumors.

Exposure to the ubiquitous air pollutant ozone produces neuroendocrine cell hyperplasia in monkey lungs (Castleman et al., 1981). We exposed hamsters 24 hours a day for 16 weeks to 0.8 ppm of ozone while at the same time treating them with DEN, and observed proliferation of neuroendocrine cells. Moreover, 12 out of 20 animals exposed to ozone and DEN had tumors of neuroendocrine cell origin; in hamsters exposed to DEN alone, 3 animals had pulmonary adenocarcinomas (Schuller and Witschi, 1990). We have thus shown that we can produce the two types of lung tumors.

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The ready availability of the two models led to preliminary studies on proto-oncogene activation in hamster lung. These preliminary experiments were and continue to be conducted by Dr. Victor Oreffo in the laboratory of Dr. F. Meyers, U.C.D, with the collaboration of Dr. P. Gumerlock. Human sequence-designed oligonucleotide primers are being used to detect and to discriminate between c-Ha-ras 1, c-Ki-ras-2 and c-N-ras of hamsters and rats by polymerase chain reaction (PCR) amplification of genomic DNA. The PCR technique allows the production of a million-fold amplification of genomic DNA in several hours. PCR has been applied for analysis of expressed mRNA transcribed from Ras genes. The successful amplification of the specific ras mRNA sequences will allow discrimination of gene expression in tumors and normal tissues. To date amplification of specific ras mRNA sequences have been performed in normal fresh tissue. The polymerase chain reaction products are now currently being used for sequence analysis of these genes in both the hamster and rat. Human allele-specific oligonucleotide (ASO) probes have already been used in hamsters at non stringent conditions. Preliminary results indicate homology is evident for N, K, and H-ras genes. We are currently in the process of sequencing these genes in the hamster. Once the sequence is known, it will be possible to obtain PCR products from tissues obtained by other means (fixed by formalin or alcohol, for example). Furthermore with the specific sequences known, it will also be possible to construct ASO probes. These probes will permit the precise identification of these activating mutations by hybridization under stringent conditions. Work has advanced to a stage where it is possible to present at a scientific meeting **(Appendix 1)**.

An exploratory study to look for proto-oncogene activation of SCLC in hamster lung (induced by subcutaneous injections of DEN and exposure to hyperoxic oxygen) is being conducted by Dr. Han-Sun Nin in our laboratory using viral oncogene probes. His preliminary studies have shown that in animals given 10 weeks of treatment, there is an increased expression of myc and fos RNA in animals given oxygen alone. A greater increase in the expression of these genes is seen in animals exposed to DEN and oxygen when compared to untreated animals or animals that have been treated with DEN alone and kept in ambient air.

These preliminary experiments suggest that it is feasible to characterize tumors produced in hamsters with techniques of modern molecular biology. The study proposed should expand substantially on these findings. DNA from treated animals will be probed with cDNA-specific oncogene probes (as opposed to viral-specific oncogene probes as is the case in the above study). Oncogene activation will be examined for multiple time points in the progression of the tumors (from early to late stages of the disease). Furthermore, comparative studies will examine the differences in the two tumor types, SCLC and non-SCLC.

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Qualifications of investigators

Dr. Hanspeter Witschi is an experienced toxicologist and has published extensively in the field of acute and chronic lung injury. He is one of the original discoverers of the hamster model for SCLC. He also has done work himself and published several papers dealing with quantitative aspects of cell proliferation in the lung.

Dr. Victor Oreffo was trained as a biochemist/cell biologist at University College, Cardiff, UK. In 1988 he came to UC Davis as a postdoctoral fellow in the laboratory of Dr. Witschi. For the past year he has worked in the laboratory of Dr. F. Meyers at UC Davis Medical Center, under the direct supervision of Dr. Paul Gumerlock, in order to learn techniques in molecular biology.

The PI and Dr. Oreffo will get additional technical expertise and help, if necessary, from:

Dr. Fred Meyers, Associate Professor, Division of Hematology and Oncology, Dept. of Internal Medicine, UC Davis Medical Center. He has an active ongoing research program with emphasis on studies on the molecular biology of human cancers.

Dr. Paul Gumerlock is an Assistant Research Geneticist and Molecular Biologist in the laboratory of Dr. Meyers and a consultant to Cetus Corporation, Emeryville, CA. He has an ongoing active research program in molecular biology, particularly the application of the PCR technique and the expression of the ras oncogene in human malignancies. He will provide direct help in application and refinement of molecular biology techniques.

Dr. Charles G. Plopper has published extensively in the field of lung structure and function and is an internationally recognized expert in pulmonary morphology. He has done extensive studies in the field of immunocytochemistry including the localization of various forms of cytochrome P450 in the lung. He will assist in the development of optimal procedures for identification of neuroendocrine cells with specific stains will and provide advice on standardization of quantitative measurements.

Dr. Jerold A. Last was originally trained as a biochemist and molecular biologist and is at present leader of the Respiratory Disease Unit at the California Primate Research Center. He is also Vice-Chair/Research, Dept. of Internal Medicine. He will add both his expertise as an experimental toxicologist/biochemist and as an administrator, facilitating collaborative efforts between different laboratories.

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11. Experimental plan and methods

General Approach

In order to produce the two different tumor types in hamster lung, we will follow a protocol that has been found to be highly reliable and reproducible (Schuller et al., 1990; Appendix 2). Non-small cell lung cancers will be produced in Syrian Golden hamsters treated, three times a week, with subcutaneous injections of 12.5 mg/kg of NNK. With this treatment we expect an 80% (or greater) tumor incidence within 6 months. In order to produce small cell lung cancers, hamsters will be injected three times a week with NNK and will also be exposed continuously to 70% oxygen in the inspired air. This regimen is expected to produce a 70%-80% incidence of small cell lung cancers within 4 to 6 months.

In each experiment, we will use 4 groups of animals: hamsters treated with NNK alone, to produce non-small cell lung cancer; hamsters treated with NNK and oxygen, to produce small cell lung cancers, and two control groups, consisting of animals exposed to oxygen and given trioctanoin injections or exposed to air and treated similarly (solvent controls). Group sizes will be 6-10 animals per treatment per time point; the exact number will depend upon preliminary data to be collected and upon consultation with our biostatistician. For the sequential analysis of oncogene expression and of neuroendocrine cell hyperplasia, we will select the following time points: 3, 6, 9, 12 and 15 weeks after beginning the treatment. In order to study oncogene expression in tumors, we will kill animals whenever they show external signs of tumor burden, such as rapid weight loss, cachexia and labored breathing (Schuller et al., 1980). For the molecular biology studies, the areas of interest such as tumor, trachea, normal lung parenchyma, eventually small bronchioles isolated by microdissection as described by Plopper et al.(1983) will be excised and flash-frozen in liquid nitrogen. For morphologic evaluation of selected lung areas, tissue will be fixed in neutral buffered formalin, a procedure that will allow analysis by conventional histopathology and by immunocytochemistry, or in Bouin's fixative.

Animals

Male Syrian Golden hamsters, outbred, will be purchased from our usual source (Charles River). When received, animals will be kept for 2 weeks in quarantine in our AAALAC-approved animal quarters; they will have access to normal lab chow and water ad libitum both during quarantine and during the experiment. Health checks (serology and histopathology) on selected animals will be done through the office of the Campus Veterinarian.

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Chemicals

NNK will be purchased from Chemsyn Science Laboratories (Lennexa, Kansas).

Oxygen exposures

The animals will be exposed to oxygen in plastic oxygen tents, as described previously (Lindenschmidt et al.,1986). An air mixture will be delivered into the chamber at a flow rate of approximately 10 l/minute; the desired oxygen concentration of 70% will be achieved by mixing 100% oxygen with compressed air. Oxygen concentrations in the chamber will be monitored with a portable oxygen meter. A carbon dioxide absorbent will be placed in the chamber. The chamber temperature is maintained at room temperature (average 72°) and the humidity is kept at 50-70%.

Tissue Processing for morphologic, biochemical, and molecular evaluation.

At the end of the experiments, the animals will be killed by deep pentobarbital anesthesia. For morphologic evaluation, lungs will be fixed in situ by perfusion with either neutral buffered formalin or with Bouin's fixative).

For biochemical analysis of tissue, normal and neoplastic tissues will be isolated by microdissection. Microdissection allows for precise and localized retrieval of tissue (this skill is regularly used in this laboratory for the isolation of mouse bronchioles). Collected tissue will be frozen at -70°. Some tissues will also be fixed in ethanol to allow retrospective studies as appropriate probes become available.

Molecular Biology studies.

DNA amplification studies. DNA will be transferred by Southern blotting and probed with appropriate ^{32}P labelled probes.

RNA amplification studies. RNA will be transferred by Northern blotting and probed with appropriate ^{32}P labelled probes.

Ras ASO probing. cDNA will be amplified by use of PCR with hamster-designed ras primers. These products will be hybridized with hamster probes for N, K, and H-ras to look for mutations in treated tissues.

DNA/RNA Extraction. DNA will be extracted from tissues using a modification of the method of Maniatis et al. (1982). Pulverized frozen tissue specimens are added to the lysis mixture (1.5 ml TE [20 mM Tris-HCL, 2 mM EDTA, pH 7.5], 0.5% SDS, proteinase K to 100 µg/ml), the mixture is incubated for 60 minutes at 65°C, and DNA

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is extracted first in an equal volume of phenol, secondly in phenol/chloroform (1:1), and finally in chloroform/isoamyl alcohol (24:1). The aqueous layer is separated by centrifugation at 3000 g for 15 minutes, and adjusted to 0.3 M Na acetate, then DNA is precipitated with two additional volumes of cold 100% EtOH at -20°C for one hour. The DNA is pelleted by centrifuging at 3000 g at 4°C for 30 minutes and the supernatant is decanted off. The pellet is washed once with 1 ml of 70% ETOH (cold), spun for 30 minutes at 4°C, the supernate decanted, and the pellet is dried in a rotary vacuum desiccator, then suspended in 1/10 TE. The DNA is quantified by UV absorbance spectrophotometry (1.0 O.D. =50 μg/ml), and stored at 4°C.

RNA will be prepared from tissues using modifications of previously described acid guanidinium thiocyanate-phenol-chloroform extraction methods (Maniatis et al., 1982; Chirgwin et al., 1979). For RNA extractions, guanidinium thiocyanate (GITC) solution is used to denature proteins and inhibit ribonucleases; the resultant lysate is centrifuged through a CsCl density cushion. GITC (5M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, pH 7.0) is prepared with 5% B-mercaptoethanol (GITC-ME) just before use. Frozen tissue is pulverized to powder in liquid nitrogen, and ground twice in GITC-ME in a mortar. 3 ml of this lysate is layered onto the CsCl in 14 X 95 mm polyallomer tubes (Beckman Laboratories). The CsCl gradient is prepared by layering 4.5 ml 2.4 M CsCl in TE onto 5.0 ml of 5.7 M CsCl in TE. The gradients are run at 35K (Beckman, SW-40 rotor) at room temperature for 15-18 hours.

The RNA pellet is suspended in 500 μl TE-SDS (10 mM Tris-HCl, EDTA, pH 7.4, with 0.5% SDS) in an Eppendorf tube prior to phenol-chloroform extraction. Two phenol/chloroform extractions are performed; an equal volume of phenol (TE saturated) and chloroform:isoamyl alcohol (24:1) is added to the RNA solution, and vortexed vigorously for 10 seconds. The phase containing RNA is removed from the protein present in the interphase and phenol phase. In a 2 ml microcentrifuge tube, the RNA is precipitated by addition of 5M NaCl to create a final NaCl concentration of 0.3 M, and two volumes of cold 100% ethanol are added. The mixture is cooled to -70°C for one hour. After cooling, the RNA precipitate is microfuged at 4°C for 15 minutes, the supernatant is decanted, and residual liquid is removed in a vacuum desiccator. After resuspension of the RNA in TE (without SDS), the RNA precipitation is repeated. This resulting RNA pellet is suspended in 100 μl of 0.2 X TE. The concentration of RNA in μg/μl is determined by the optical density at 260 in a spectrophotometer.

<u>mRNA Expression:</u> cDNA sequences which correspond to different mRNAs will be amplified according to the method of Kawasaki et al.(1988) with the modification of Noonan and Roninson (1988). In this procedure, the extracted cellular RNA is converted to cDNA by reverse transcription using a random hexadeoxynucleotide primer

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for reverse transcription. One microgram of total RNA is used in the reverse transcription utilizing 100 pmol of random primer (EK-293), 20 U RNasin (Promega Biotech), 0.5 mM each of the dNTPs (dATP, dCTP, dTTP, and dGTP) and 200 U Moloney Murine Leukemia Virus (Mo-MuLV) reverse transcriptase (Bethesda Research Laboratories) prepared in PCR buffer (50 mM KCl, 20mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, and 100 µg/ml Bovine Serum Albumin (BSA). For the reverse transcription, the mixture is mixed well, pulsed in a microfuge, and incubated at room temperature for 15 minutes followed by 42°C for 30 minutes. The mixture is cooled to room temperature, pulsed in a microfuge to the condensate, and 2 µl is subsequently used for RNA/PCR.

PCR Reactions using cDNA: PCR will be carried out essentially as described by Saiki et al.(1985) using recombinant thermostable DNA polymerase originating from Thermophilus aquaticus (rTaq). Primers are used at 10 pmoles each, dNTPs are reduced to 1µl of the 10 mM stock, rTaq enzyme is used at 2U per reaction in a total of 100 µl reaction. The substrate for RNA/PCR is 2 µl (100ng) of the 20 µl cDNA prepared.

PCR profiles of 95°C for 1 minute (denaturing of double strands), 55° for 30 seconds (annealing of amplimers), and 72° for 30 seconds (synthesis of DNA) are performed in a programmable heat block (Perkin Elmer-Cetus) for 30-50 cycles.

PCR products are screened by running 9 µl of the reaction mixture in 2% NuSieve (FMC, Rockland MD), 1% agarose gels in Tris -borate EDTA buffer (TBE). For size markers, the 123bp DNA ladder (Bethesda Research Laboratories) is used. Gels of 75 ml are run in wide mini-sub cells (Bio-Rad Laboratories) in TBE at a constant 110 volts for approximately 90 minutes. Gels are stained in an ethidium bromide solution (0.5 µg per ml) for 30 minutes, destained for 30 minutes, and photographed under ultraviolet light with a polaroid Land camera

ASO Probing of RNA/PCR, DNA/PCR Products: RNA/PCR or DNA/PCR products to be probed are run in 2% agarose gels in a Tris-borate EDTA electrophoresis buffer (TBE) in a mini-gel system. Alkaline transfer to Zeta-Probe nylon filters (Bio-Rad Laboratories) in a wick-action transfer is done with a 0.4 N NaOH solution in water. Transfers are allowed to proceed for 90 minutes. After transfer, blots are neutralized in 2 X SSC for 5 minutes. Blots are prehybridized in a solution of 3 M tetramethyl ammonium chloride (TMAC), 50 mM Tris-HCL pH 7.5, 2 mM EDTA, 5 X Denhart's solution, and 0.3% SDS at 55° for 1 hour with circular agitation. Hybridization with ASO probes that are kinase-labelled with gamma-^{32}P-ATP is carried out in 5 ml of the TMAC buffer with $2x10^6$ cpm per ml of probe added. Hybridization is continued at 55°C for 1 hour. The hybridization buffer and the first wash of 2x SSC with 0.1% SDS (50 ml) at room temperature are discarded as radioactive waste. A second wash is carried out at room temperature with 2x

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SSC with 0.1% SDS. Blots are quickly rinsed in the TMAC buffer minus the Denhart's solution and extensively washed in the same buffer at 61°C for 1 hour. Blots are blotted dry with Whatmann 3MM paper and exposed to Kodak XAR film for 1 hour at -70°C. The film is then developed in an automatic processor.

RNA slot blot. Whole cellular RNA in various amounts (1-10 µg) from each sample is taken for slot blot analysis. The RNA is brought up to a total volume of 50 µl for each concentration in TE buffer, then 30 µl of 20 X SSC and 30 µl of 37% formaldehyde are added to each sample. The samples are incubated at 60°C for 15 minutes, placed on ice, and then loaded onto individual wells on a Schleicher and Schuell Minofold II slot-blotter assembled with a BA85 nitrocellulose membrane. Each well is washed with 200 µl of SSC. The membrane is placed in a vacuum oven at 80°C for 1-2 hours.

The membrane is prehybridized in 5 X SSPE(1x SSPE = 150 mM NaCl, 10 mM Na phosphate, 1 mM EDTA); 5 X Denhardt's; 50% formamide; 0.1% SDS and 200-400 µg/ml of sheared salmon sperm DNA overnight at 42°C. The membrane is then hybridized with the same buffer without the DNA and containing the probe of choice for 12-16 hour at 42°C. The membrane is then washed, placed against film in an x-ray cassette for 12-48 hours and then developed. V-onc probes are purchased from Oncogene®.

Quantitation of pulmonary neuroendocrine cells

Several methods (histopathology, immunocytochemistry, electron microscopy) are available to visualize and to count the number of pulmonary neuroendocrine cells and of neuroepithelial bodies. For the purpose of our experiments, we will use two methods which allow us to identify cells under the light microscope: a silver stain (Grimelius method) and an immunstain for calcitonin. Both methods have been used in experiments similar to our proposed ones (Taylor, 1977; Kleinerman et al., 1981; Tabassian et al., 1989).

Lungs will be fixed by the method proposed by Pack et al. (1986). Animals will be killed by an overdose of pentobarbital. The thorax will be opened. The deflated lungs will be clamped and immersed in Bouin's fluid to harden (20 to 60 min). They then will be sliced in 1 mm slices and reimmersed in the same fixative for up to 24 hours before embedding. According to Pack et al., consistent results will be obtained with the Grimelius (1968) method. Whenever a silver stain is performed, positive control tissues (pancreas) will be included. We propose to prepare 10 sections from each lung with a minimum of 100 um between each section (Pack et al., 1986).

For immunocytochemistry, we will use commercially available anti-calcitonin as the primary antibody. The avidin-peroxidase

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method will be applied by using the Vectastain ABC-Kit (Vector Laboratories, Burlingame, CA). The procedure has been used in our previous experiments (Schuller et al., 1988). If necessary, the procedure will be modified as described by Tabassian et al. (1989). Thyroid gland will serve as a positive control tissue for quality control of the stains. Both procedures will be established and, where necessary, modified and improved in close collaboration with Dr. Charles Plopper, an experienced morphologist and immunohistochemist.

Several procedures have been described in order to quantitate neuroendocrine cells in the lungs of experimental animals: determination of number of groups or of individual argyrophilic cells/square cm by unit area (Taylor, 1977; Linnoila et al., 1984; Pack et al., 1986) or by unit airway length (Kleinerman et al., 1981). At present, we favor the method which will provide number of cells per unit area; this would allow us to include cells located in the pulmonary parenchyma and not limit the count to airway cells only. This is important because we have recently shown that, in hyperoxia, cells located in the parenchyma may transdifferentiate into neuroendocrine cells and, presumably, give rise to tumors (Schuller et al., 1990). In order to develop the most satisfactory method for our purposes, we will seek the expert technical advice of Dr. Kent Pinkerton, a recognized expert in morphometry.

Analysis and interpretation of data

At the moment, we cannot yet predict the quantitative or qualitative nature of differences to be found between the different treatment groups with regard to oncogene expression. However, from the literature that exists on human cell line studies and fresh tissue studies, certain oncogenes are expected to be associated with a given tumor type. Therefore we expect the following findings:

SCLC:	myc expression should increase
	Rb expression should decrease
	Wildtype p53 gene expression should also decrease.
non-SCLC	ras mutation expected (especially K-ras)
	Rb expression may decrease
	Altered myc expression.
	Wild type p53 gene expression also decreased.
	Mutated form may cooperate with ras in transformation.

Little is known about the stages in tumorigenesis in which a given proto-oncogene is activated/deactivated. This study will begin to define the temporal patterns of expression. We anticipate that Drs. Meyer and Gumerlock, experienced in studies on oncogene expression, will assist in evaluation of the data. For analysis and for the numerical evaluation of neuroendocrine cell proliferation,

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10 sections per lung from each animal will be evaluated and the number of cells counted will be noted. For each group ($n = 10$), mean and S.D. will be calculated. Differences between any single experimental and control group will be compared by t-test, with the level of significance set at $p < 0.05$; comparisons between different groups will be done by analysis of variance. If necessary, more complex statistical analyses will be performed in consultation with a biostatistician.

Timetable for proposed studies

We plan to meet the objectives of our proposed experiments within a three year period. During the first year, we will produce non-small cell and small cell lung cancers in experimental animals and will examine fully formed tumors with our molecular probes. According to our preliminary observations and data, tumors will develop within a 4 to 6 month period. While tumors develop in vivo, we will finish our preliminary work on the characterization and preparation of the hamster-specific molecular probes required for tumor characterization. Also during the first year we will develop methods for quantitation of neuroendocrine cells (development of immune stains, counting procedures) and begin the study on neuroendocrine cell proliferation at different time points after treatment begins.

In the second and third years, we will continue the studies on cell proliferation. We also will examine lung tissues from hamsters with our molecular probes at earlier times than are required for tumor development. Specifically, we plan to establish an oncogene profile in peripheral lung tissue, small airways and tracheae in animals which have been exposed to a carcinogenic regimen producing non-small cell and small cell cancers (tumor development in 16-24 weeks) for 3, 6, 9 and 12 weeks. This experiment will specifically examine the hypothesis that specific profiles of oncogene expression might predict the nature of tumors that will eventually develop.

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12. AVAILABLE FACILITIES AND RESOURCES

Facilities and resources

The proposed work will be performed on the campus of the University of California, Davis (UCD), in various laboratories at the Institute of Toxicology and Environmental Health (ITEH) and University of Davis Medical Center (UCDMC) research laboratories in Sacramento. The ITEH has approximately 60,000 square feet of laboratory space. Facilities at ITEH and UCDMC are well equipped with modern instrumentation and staffed by experienced scientists and technologists. ITEH has complete inorganic chemistry, organic chemistry, biochemistry and cell biology facilities. A complete mechanical and maintenance shop for repair of equipment, a complete electronic shop for development of electrical and electronic devices for experimental systems, light microscopy facilities, scanning and transmission electron microscopy facilities, photography laboratory and use of modern word processors. The facilities of the remainder of the UCD campus are also available as required in the course of this research.

Exposure and maintenance of animals, lung pathology, biochemistry and some molecular biology studies will be carried out in the laboratory facilities at ITEH in spaces fully equipped for this purpose. Laboratory: At UC Davis (Institute for Environmental Health Research) Exposure room, 150 square feet, containing four 450 liter plastic inhalation chambers for exposure to hyperoxic and hypoxic atmospheres, including an oxygen meter for spot sampling and analysis of chamber atmospheres. Six hundred square feet of general laboratory space, equipped with animal and analytical balances, general purpose centrifuge and spectrophotometer, and facilities to collect through appropriate fixation and to store tissue specimens.

Histopathology: fully equipped histopathology laboratory at the ITEH facility.

Computer: Macintosh and PC-type desk-top computers available for sole use by PI.

Animals: Six hundred square feet of animal holding space equipped with stainless-steel animal racks and plastic animal cages, AAALAC approved.

The majority of the molecular biology studies will be performed at UCDMC. The major equipment for these studies includes: 3 Sorvall model RC2-B high speed refrigerated centrifuges; several bench top refrigerated centrifuges; 2 Beckman LS-50E ultra-centrifuges; Packard Tri-carb 3375 liquid scintillation spectrometer; Beckmann LSC; programmable heat block (DNA thermal cycler) Perkin Elmer Cetus PCR machine; Dionex HPLC; Perkin-Elmer fluorescence spectrophotometer 204; Beckman spectrophotometer; 219

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Cary dual beam spectrophotometer (Varian); analytical balances; pH meters; BioRad DNA sequencing apparatus Sequi-gene™; BioRad electrophoresis gel boxes; 2 cold rooms (1 at each site) for the preparation of fractions from animal tissues; light microscopy with wild M-8 stereo microscope; Zeiss research microscope and other general lab functions.

Other support available to H.P.Witschi:

1. Currently active support

a. NIH 2 PO1 ES 00628-17 - **Pulmonary effects of environmental oxidant pollutants** (PI C.G. Plopper)
10%
1-1-1989 to 12-31-1994
$ 477,888 total; 48,606 subproject
Studies on modification of lung tumor development, measured by cell kinetic analysis, in mice exposed to ozone
No overlap
No modifications

b. NIH CA 46496 - **Cancer prevention by protease inhibitors** (PI Ann R. Kennedy)
20%
9-1-87 to 8-31-1990
$ 91,000 subproject
Studies on modification of lung tumor development in mice by protease inhibitors
No overlap
No modifications

c. Health Effects Institute, Cambridge, MA - **Air pollutants and neuroendocrine lung cancer**
10%
1-1-1989 to 6-30-1990
$ 193,531
A study to examine whether ozone or NO2 in conjunction with DEN produces neuroendocrine lung cancer in hamsters
No overlap
No modifications

2. Applications pending review

The competitive application of NIH CA 46496 has been reviewed and approved; however, as of to date no funding decison has been made.

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13. Budget justification

First period

Personnel

H.P. Witschi will be responsible for the overall supervision of the project. He will design and oversee exposure protocols, coordinate sampling and preparation of lung tissues and serve overall as study director. He also will analyze or oversee analysis of lung slides in the cell proliferation studies.

V.I. Oreffo will conduct all experiments relating to the study of oncogene expression in lungs and tumors. He is at present working in the laboratory of Dr. Gumerlock where he is learning all necessary techniques and procedures. It is anticipated that in another 6 months he will be fully capable to carry out all aspects of the proposed research. No salary is requested for him; he was recently notified that a his salary is being provided for by a fellowship award from other programmatic funds.

Laboratory technician: will conduct studies in animals designed to produce tumors and, at the end of the experiment, perform autopsies and take care of tissue sampling and preparation.

Histology technician: will embed, cut and stain lung samples; will be trained in special staining techniques and in procedures involving quantitation of pulmonary neuroendocrine cells.

Supplies and Expense. Costs for histology, including the purchase of necessary antibodies and reagents for doing immunocytochemistry are budgeted at $8,000/year. Cost for molecular biology experiments (reagents) at $9,500/year.

Animal costs are budgeted on purchase of 200 hamsters ($5.50 each) and their maintenance for an average of 6 months, at the current UC Davis rate of $ 0.38/day per animal.

Travel. Funds are requested to attend 1 scientific meeting each for Dr. Witschi and Dr. Oreffo.

Equipment. A Polymerase Chain Reaction Machine is at present available only in Dr. Meyer's laboratory at UC Davis Medical Center, Sacramento, and is used to its fullest. To carry out the proposed work, an identical machine is required on site at Davis. Second and third year equipment budget is predominantly for purchase of replacement parts for existing equipment (oxygen meter, oxygen tents).

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2nd and 3rd periods

No additional equipment is requested. Personnel costs are increased 6% per year to cover cost-of-living and merit increases; supplies and other expenses are increased 6% per year for inflation. The total number of animals purchased will remain essentially the same, purchase and maintenance of rats being at a similar cost to hamsters.

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14. Biographical Sketches

Hanspeter Witschi, MD
Education: Universities of Bern and Geneva, Switzerland (MD 1960)
Present position: Professor, University of California, Davis

Diplomate, American Board of Toxicology (DABT)
Diplomate, Academy of Toxicological Sciences (DATS)

Research and Professional Experience:

1961-1964	First Assistant, Institute of Forensic Medicine, Bern University, Switzerland
1965-1966	Toxicology Research Unit, British MRC, Carshalton, England
1967-1969	Research Associate, Dept. of Pathology, University of Pittsburgh, PA
1969-1977	Assistant to Associate Professor, Dept. of Pharmacology, Faculty of Medicine, University of Montreal, Canada
1977-1981	Senior Research Staff Member II, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
1981-1987	Section Head, Toxicology Section, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
1987-Present	**Professor of Veterinary Pharmacology/Toxicology and of Internal Medicine, UC Davis; Associate Director, Toxic Substances Research and Teaching Program, University of California, Davis**

1976-1977	Member, Research Committee, Canadian Thoracic Society
1980-Present	Member, Scientific Review Panel for Health Research, EPA, Office of Research and Development
1980-1984	Member, Toxicology Study Section, DRG - NIH
1986-1990	Member and Chairman (1989/90), Environmental Health Sciences Review Committee, NIEHS.
1988-1992	Board Member, American Board of Toxicology (ABT)
1978-1986	Associate Editor, Toxicology and Applied Pharmacology
1978-Present	Editor USA, Toxicology

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PUBLICATIONS (total: approx. 150, dating back to 1958)

Schuller H.M., Witschi H.P., Nylen E.,Joshi P.A., Correa E. and Becker K.L. Pathobiology of lung tumors induced in hamsters by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the modulating effect og hyperoxia. Cancer Res. 50: 1960-1965, 1990.

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Witschi, H.P. Interpretation of early lesions in the mouse lung: Fibrogenesis and tumorigenesis, pp. 195-208. In D. Dungworth, G. Kimmerle, J. Lewkowski, R. McClellan, W. Stoeber (Eds.), Inhalation Toxicology, ILSI Monographs (U. Mohr, Ed.), Springer-Verlag, 1988.

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Schuller, H.M., Becker, N.M and Witschi, H.P. An animal model for neuroendocrine lung cancer. Carcinogenesis 9:293-296, 1988.

Witschi, H.P. Ozone, nitrogen dioxide and lung cancer: A review of some recent issues and problems. Toxicology 48:1-20, 1988.

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Last, J.A., Warren, D.L., Pecquet-Goad, E. and Witschi, H.P. Modification of ozone of lung tumor development in mice. JNCI. 78:149-154, 1987.

Lindenschmidt, R.C., Margaretten, N., Griesemer, R.A. and Witschi, H.P. Modification of lung tumor growth by hyperoxia. Carcinogenesis 7:1581-1586, 1986.

Lindenschmidt, R.C., Sendelbach, L.E., Witschi, H.P. Price, D.J., Fleming, J. and Joshi, J.G. Ferritin and in vivo beryllium toxicity. Toxicol. Appl. Pharmacol 82:344-350, 1986.

Lindenschmidt, R.C., Tryka, A.F., Goad, M.E. and Witschi, H.P. The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. Toxicology 38:151-160, 1986.

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Witschi H.P. and Morse C.C. Enhancement of lung tumor formation in mice by dietary butylated hydroxytoluene: dose-time relationships and cell kinetics. JNCI 1983; 71:859-866.

Haschek, W.M., Reiser, K.M., Klein-Szanto, A.J.P., Kehrer, J.P., Smith, L.H., Last, J.A., and Witschi, H.P. Potentiation of butylated hydroxytoluene-induced acute lung damage by oxygen. Cell kinetics and collagen metabolism. Am. Rev. Respir. Dis. 127:28-34, 1983.

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Victor Ifeanyi Chuckwuemeka Oreffo, PhD

Education: University College, Cardiff, UK, PhD, Biochemistry/ Cell Biology (1988)

Present Position: Research Associate, University of California, Davis (Experimental Toxicology)

PUBLICATIONS

Richards R.J., Davis N. and Oreffo V.I.C. (1987). Isolation, biochemical characterization and culture of lung type II cells of rat. Lung 165: 143-148.

Oreffo V.I.C., Thomas D. and Richards R.J. (1988). Comparative toxicity studies on cultured alveolar type II cells and lung fibroblasts exposed to agents that produce pulmonary oedema. CHIMIC Aoggi 1: 57-61.

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Richards R.J., Oreffo V.I.C. and Lewis R.J. Clara cell cultures from the mouse and their reaction to bronchiolar toxins. Environ. Health Persp. (in press)

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